

Genetic Interrelationships among Populations of *Gila cypha* in the Colorado River Ecosystem

Marlis R. Douglas
Michael E. Douglas

Dept. Fish, Wildlife & Conservation Biology
Colorado State University

Relationships among Populations of Humpback Chub (HBC)



Humpback Chub (*Gila cypha*)

HBC in Grand Cn

9 locations
=
aggregates



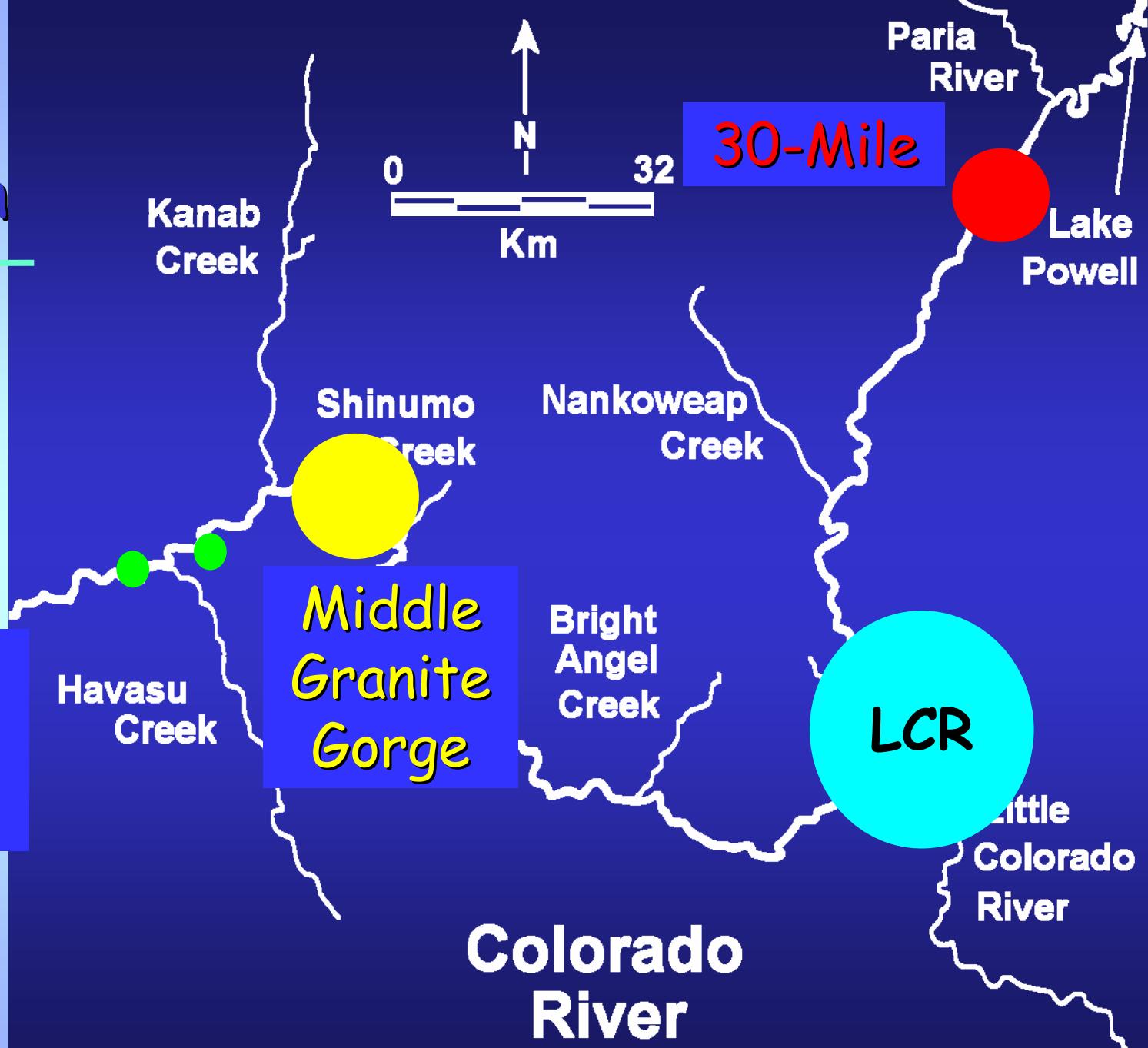
Gila cypha in Grand Canyon

- Do aggregates represent discrete populations ?
 - migration among aggregates?
 - local reproduction?
 - local recruitment?

HBC in Grand Ch

locations

Western
Grand
Canyon



Gila cypha in Grand Canyon

- Relationships among 9 “aggregates”?
- Mainstem / LCR continuum ?
 - 30-Mile vs LCR?
 - LCR vs Middle Granite Gorge
 - LCR vs Western Grand Canyon

HBC in Grand Cn

aggregates

LCR vs 30M

LCR vs MGG

LCR vs WGC

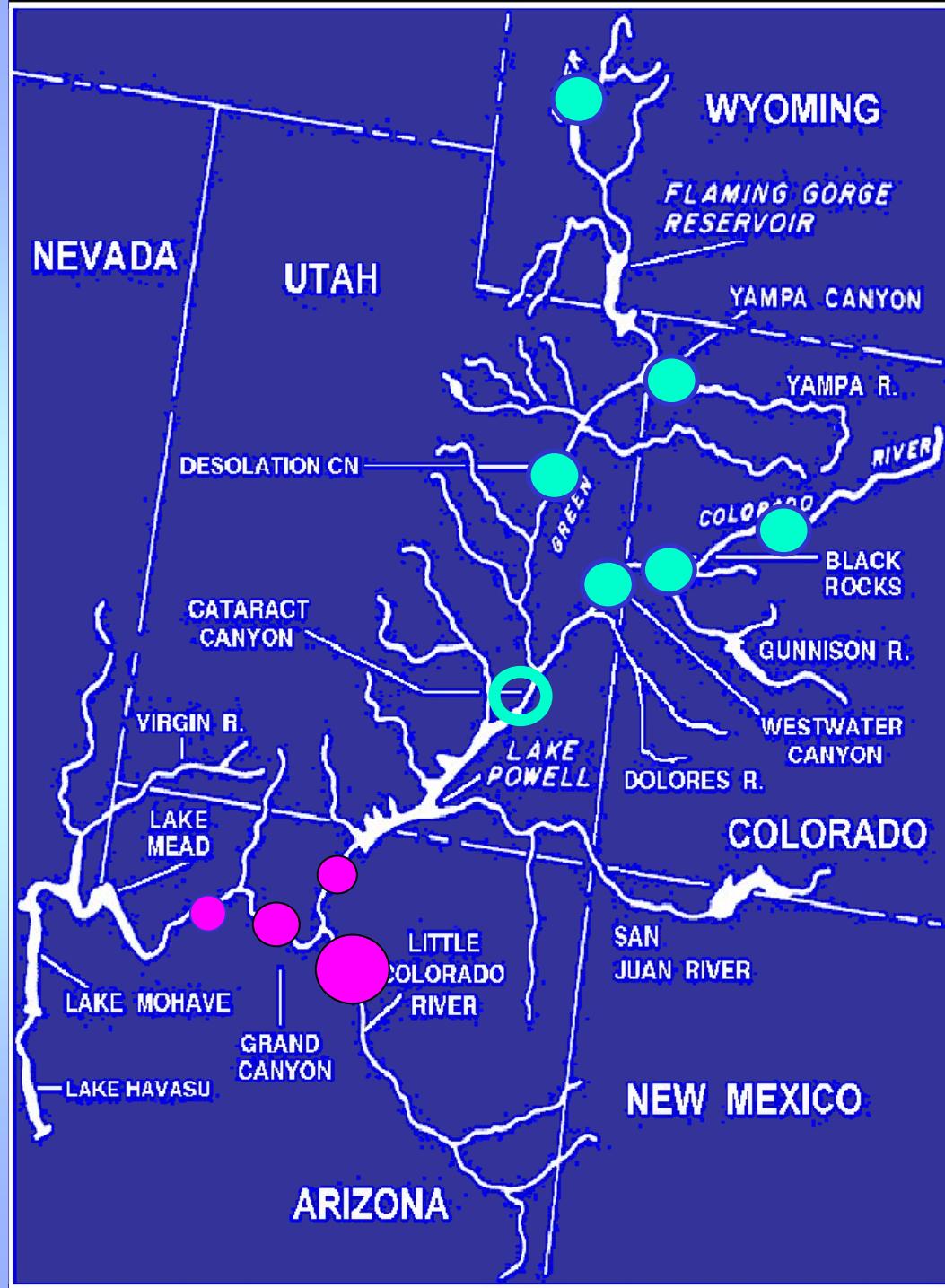


Gila cypha in Grand Canyon

- Examine population structure of *Gila cypha*
 - genetic interrelationships among aggregates in Grand Canyon
- Is there a baseline?
 - how much differentiation to expect?
 - which methods to use?

Gila cypha in Colorado River Basin

- Upper Basin
- Grand Canyon



Colorado R *Gila*



Roundtail Chub



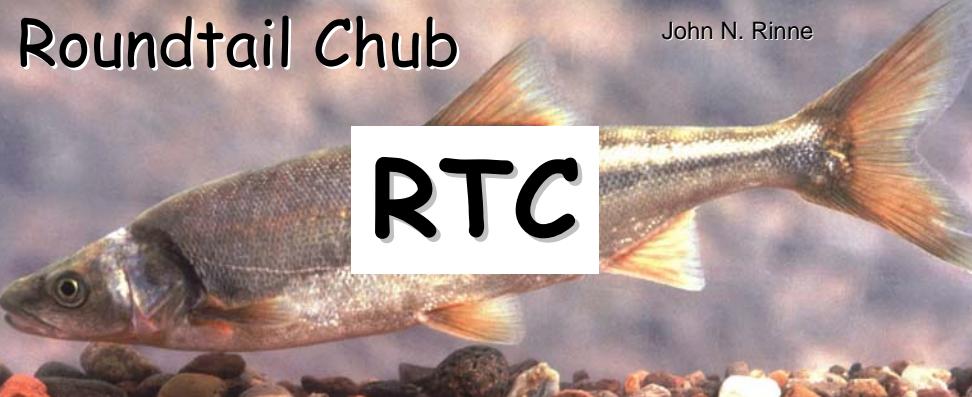
Bonytail



Colorado R *Gila*



Colorado R *Gila*



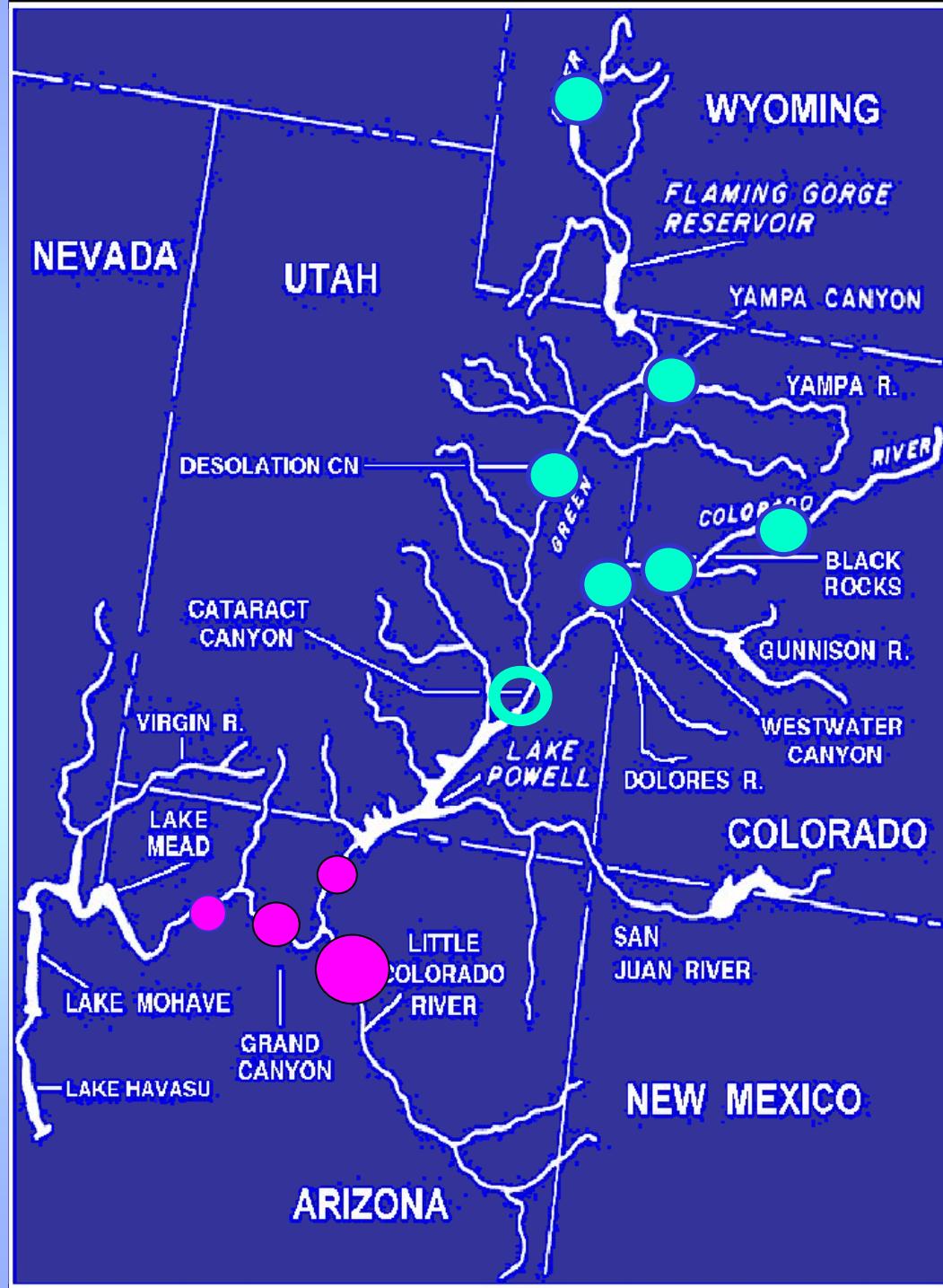
Entire Basin

Upper Basin

- HBC
- RTC

Grand Canyon

- HBC

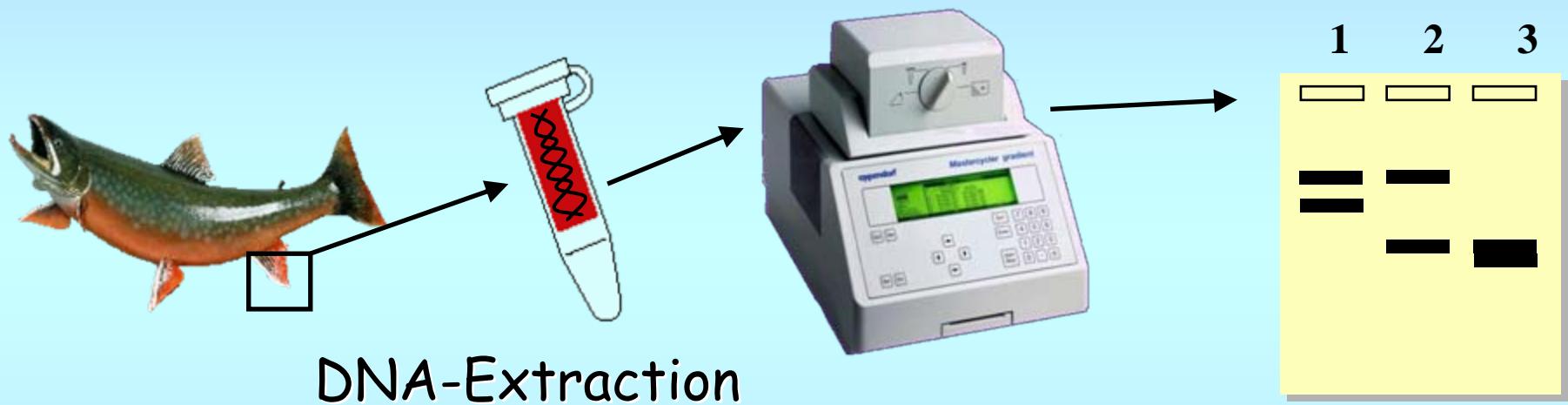


Study Objectives

- Examine population structure among *Gila* in the Colorado River Ecosystem
- **genetic interrelationships** of populations at
 - **spatial scales** (= population structure)
 - **temporal scales** (= evolutionary history)

Approach: Molecular Genetic Methods

Tissue collection



DNA-Extraction

DNA Amplification
(PCR)

Electrophoresis

Molecular Genetic Methods

- mitochondrial DNA sequence analysis
 - >> evolutionary history
 - >> long-term conservation goals (ESUs)
- microsatellite DNA analysis
 - >> population structure
 - >> management units (MUs)

Msat Loci & Sample Sizes

- high variability
 - + population-level analyses
 - # alleles >> # sample sizes
- codominant inheritance
 - + species ecology (e.g., introgression)
 - larger sample size to encapsulate pop. variation

Sampling: Grand Canyon

- 9 aggregates / 234 HBC
-

- 30-Mile Springs	11
- Little Colorado River	77
- Lava to Hance	8
- Shinumo Ck	26
- Stephen's Aisle	7
- Randy's Rock	80
- Kanab Ck	4
- Havasu Ck	11
- Western Grand Cn	10

Table 5

Sampling: Upper Basin

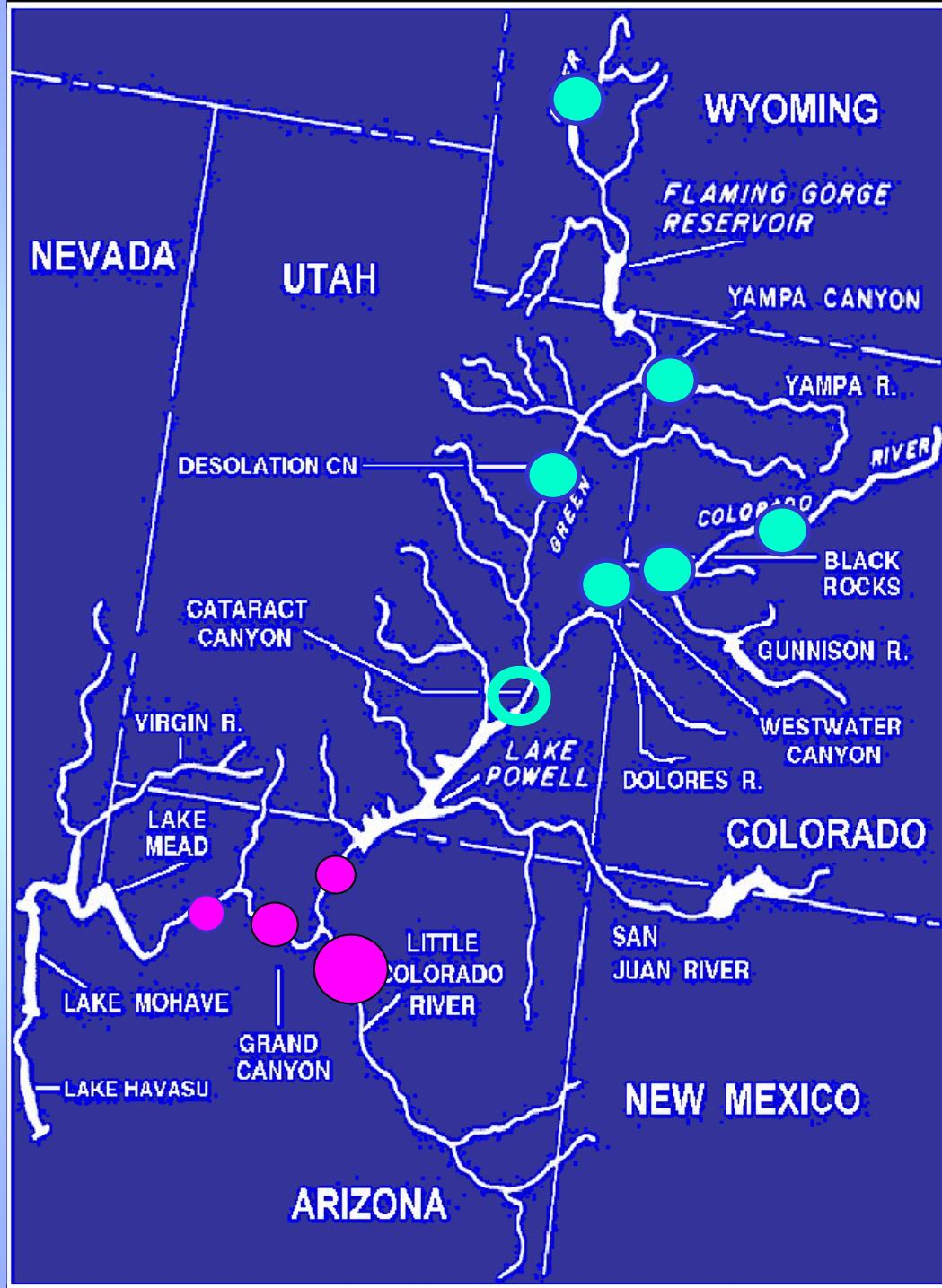
- 5 locations / 186 HBC + 208 RTC

	HBC	RTC
- Yampa R	7	56
- Desolation Cn	51	19
- Black Rocks Cn	51	43
- West Water Cn	77	59
- 15-mile Reach	-	31

Table 5

Sampling

- **Upper Basin**
208 RTC
186 HBC
- **Grand Canyon**
234 HBC
- **Broodstock**
15 BTC



Results: mtDNA Analysis

- 8 HBC/RTC with BTC haplotypes in
 - HBC Desolation Cn 2 (21)
 - HBC 30-Miles 2 (9)
 - HBC LCR 2 (46)
 - HBC Randy's Rock 2 (44)
 - RTC Desolation Cn 1 (22)

Table 2

Parsimony haplotype network

HBC - GC
HBC - UB
RTC - UB

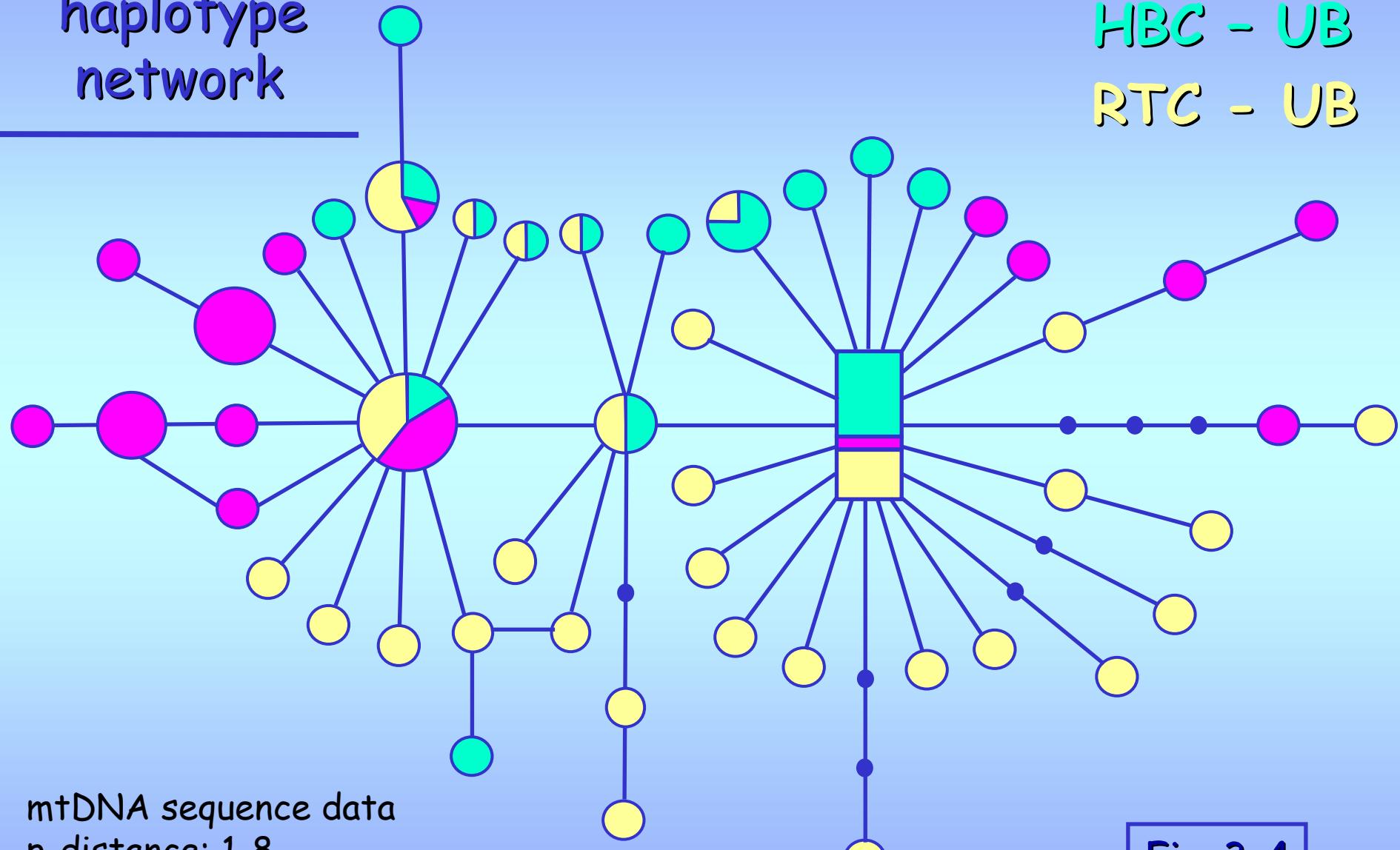


Fig. 3+4

Mitochondrial DNA Analysis

Comparison	% Sequence Divergence
HBC_{UB} / HBC_{GC}	no difference
HBC / RTC_{UB}	~ 0.1% (negligible)
BTC / $HBC + RTC_{UB}$	~ 5%
RTC_{UB} / RTC_{LB}	~ 4%
$Ptychocheilus$ / $Gila$	~ 6%

Table 3

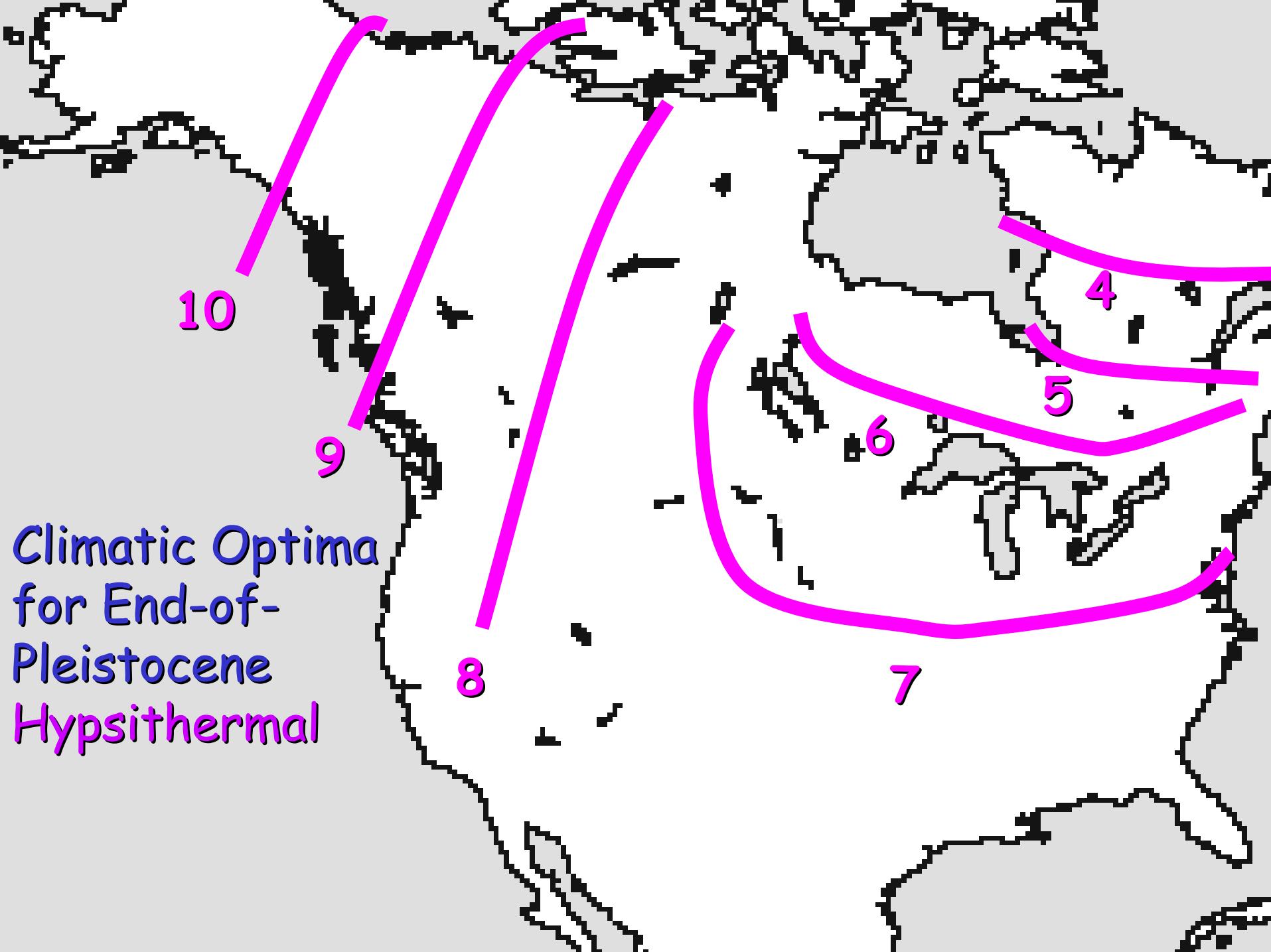
Results: mtDNA Analysis

- many haplotypes
but low divergence among haplotypes
- historic population bottleneck
thus low effective population size N_e
- population expansion since bottleneck
re-colonization of upper basin

Table 2 + 4

mtDNA + Colorado River Fishes

- Douglas, Brunner, Douglas (2003)
Freshwater Biology [PDF available](#)
- Drought in “recent” geologic time had
severe impacts on Colorado River fishes



Historic Demography & Management

- Historic Population Bottleneck
 - Low effective population size N_e
 - Shorter evolutionary time scale
 - Different molecular approaches

Results: Msat Analysis - Diversity

- High allelic diversity within populations
- Moderate to high levels of heterozygosity
- Unique alleles for populations, basins, species

Table 7 + 8, Appendix 5 + 6

Results: Msat Analysis - Diversity

- **Allele frequencies** between populations show differences (Bonferroni correction)
 - but
 - sample size effects!

Table 9

Conclusion (1)

- Data support recognition of six MUs

(1) HBC_{GC} : Marble/Grand Cn

(2) $HBC_{DES} + RTC_{DES}$: Desolation Cn

(3) HBC_{UB} : upper basin

(4) RTC_{UB} : upper basin (except Yampa R)

(5) RTC_{yR} : Yampa R

(6) BTC : Bonytail

msat - STRUCTURE: 6 Clusters

F8

HBC-UB

HBC DES

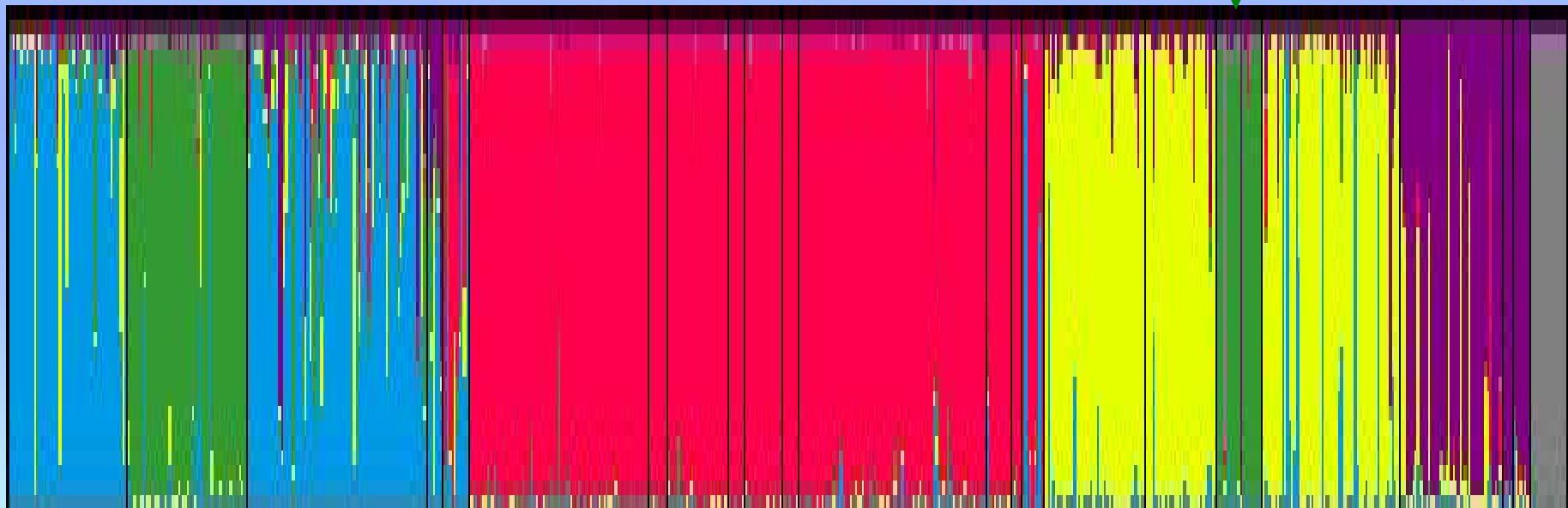
HBC-GC

RTC-UB

/ RTO
DES

BTC

RTC
YAM



Gila cypha - Upper Basin

- **Populations:**

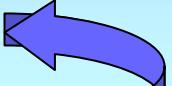
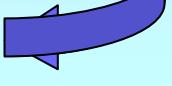
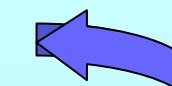
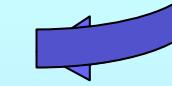
- Yampa River 
- Desolation Cn 
- Black Rocks Cn 
- Westwater Cn 
- Cataract Cn no samples

Table 9 = sig.
GP2,3,6,7

Table 9 = ns, GP1

Conclusion (2)

❖ HBC in Marble/Grand Cn genetically similar

- aggregates connected by gene flow

pairwise tests non-significant
GP5 = genepool identified by TFPGA

Table 9

- >> downstream drift of larvae + juveniles
- >> LCR likely primary source
- >> occasional local reproduction

msat - STRUCTURE: 5 Clusters

UB-H

UB-H

GC-H

UB-R

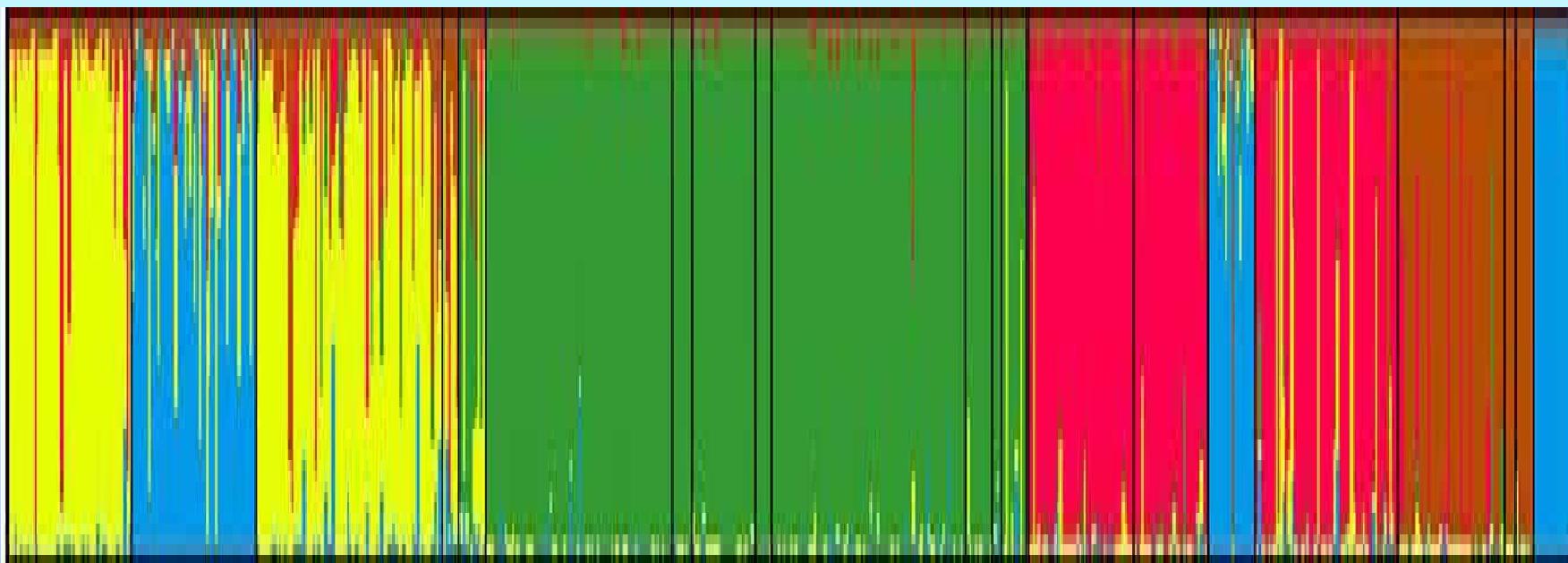
UB-R

YR-R

DC/BT

DC/BT

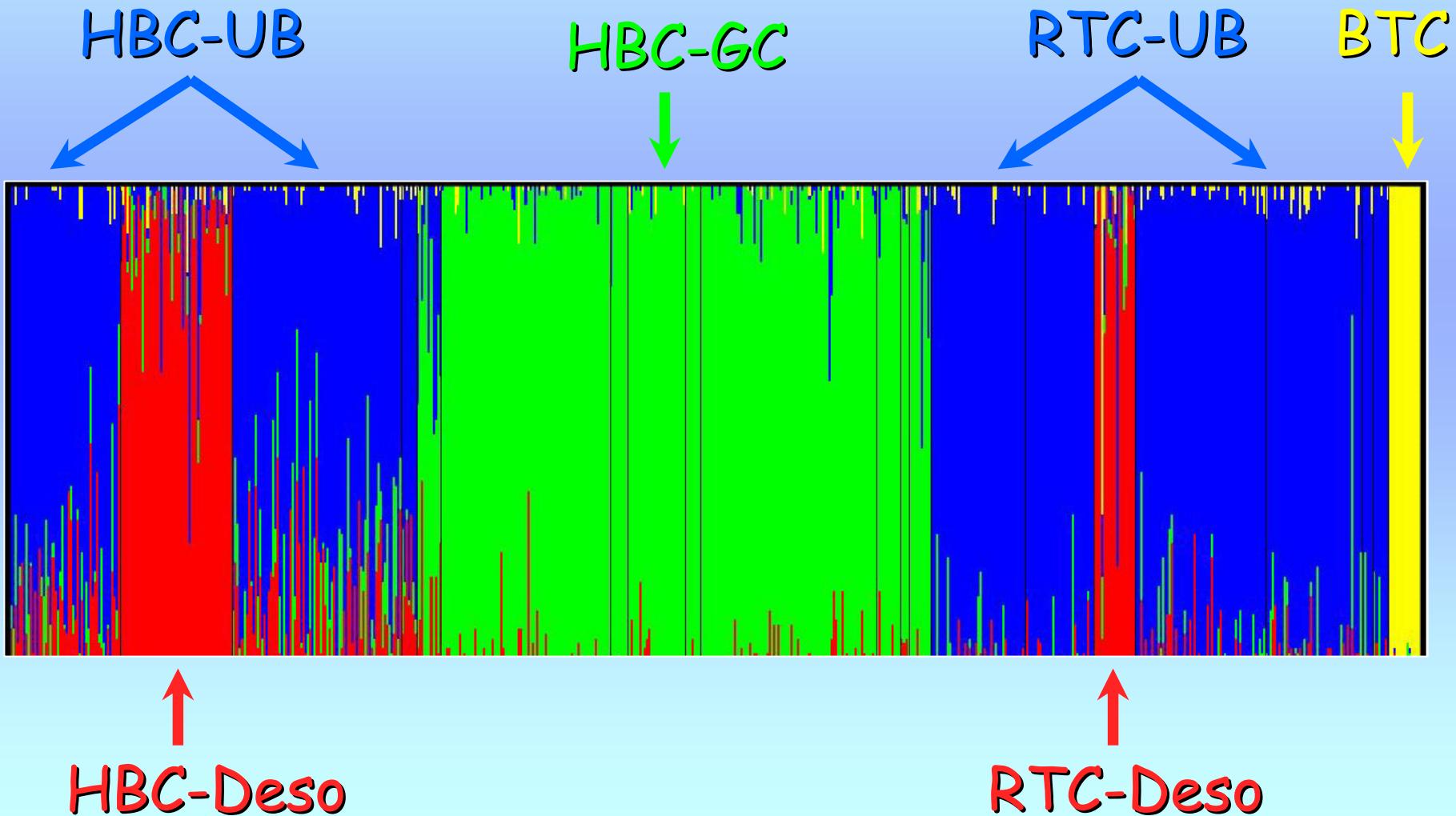
DC/BT



H	H	H	HH	H	HHH	H	HHH	R	R	R	R	R	R	B
B	D	W	Y 3	L	L S S	R	H K W	B	1	D	W	Y	Y	B
K	E	W	A 0	C	A H A	A	A A G	K	5	E	W	A	D	T
R	S	C	M M	R	H N A	N	V N C	R	M	S	C	M	T	C

msat - Bayesian Assignment Test

Four-Group Clustering (Program **BAPs**)



Conclusion (3)

❖ 30-Mile population in Marble Cn

- admixture with BTC (mtDNA)
- association with HBC_{UB}
- distinct genepool

Table 2

Table 11, Fig 8

Table 9 = GP4

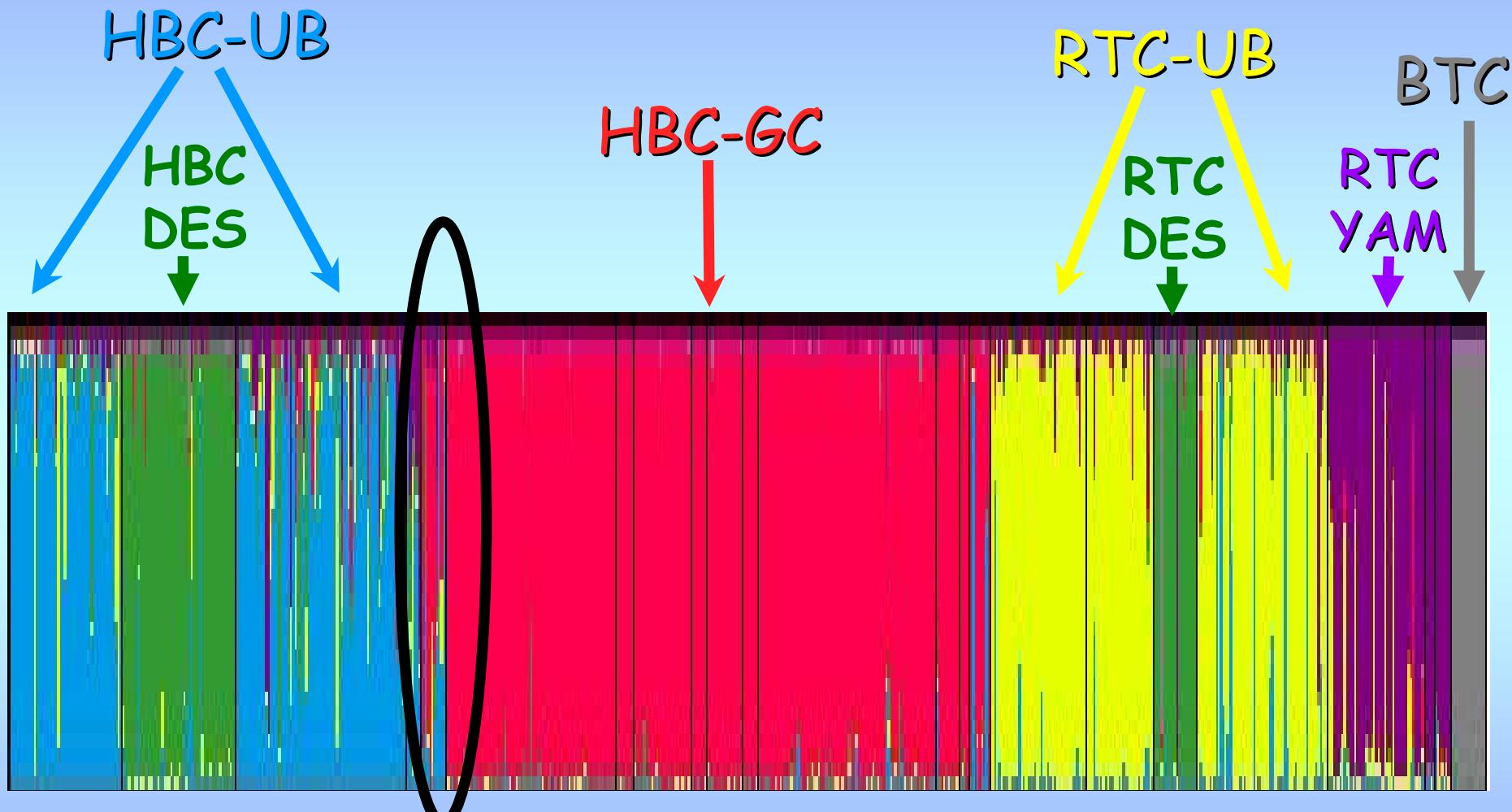
>> occasional local reproduction

Valdez & Masslich 1999

BUT - small sample size, low pop. number

msat - STRUCTURE: 6 Clusters

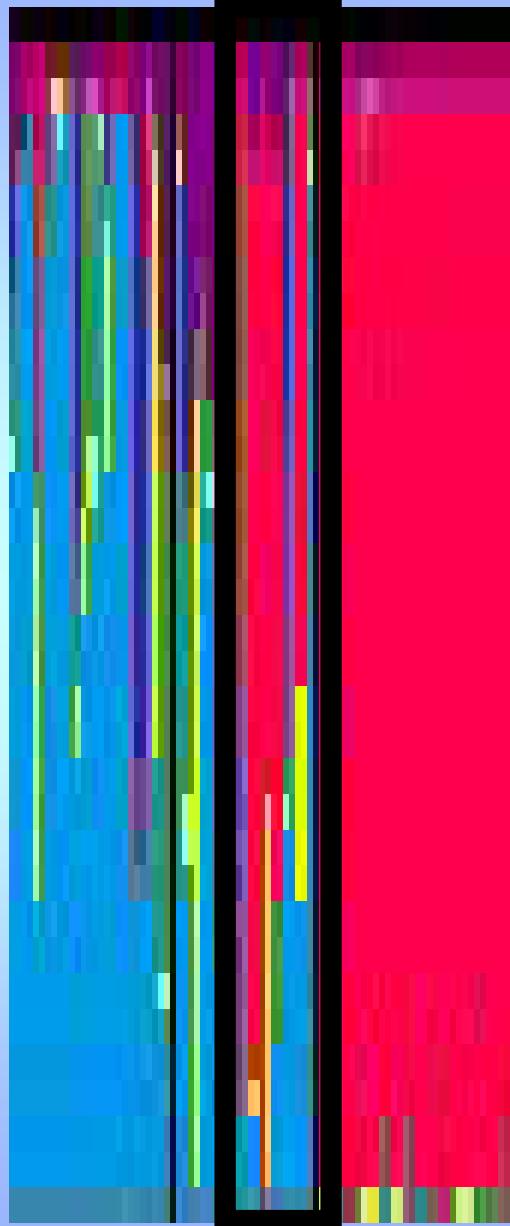
Fig 8



H	H	H	H	H	H	H	H	H	H	R	R	R	R	R	R	R	RRB
B	D	W	Y	3	L	S	S	K	KW	B	1	D	W	Y	YLB	Y	YLB
K	E	W	A	0	C	A	H	A	AAG	K	5	E	W	A	DYT	A	DYT
R	S	C	M	M	R	H	N	A	VNC	R	M	S	C	M	TCC	M	TCC

msat - STRUCTURE: 6 Clusters

F8



HBC - 30-Mile

DES
RTC_{UB}
RTC_{YR}

Conclusion (4)

❖ Upper Basin HBC / RTC

- RTC in Yampa River forms distinct cluster

Table 11, Fig 7+8

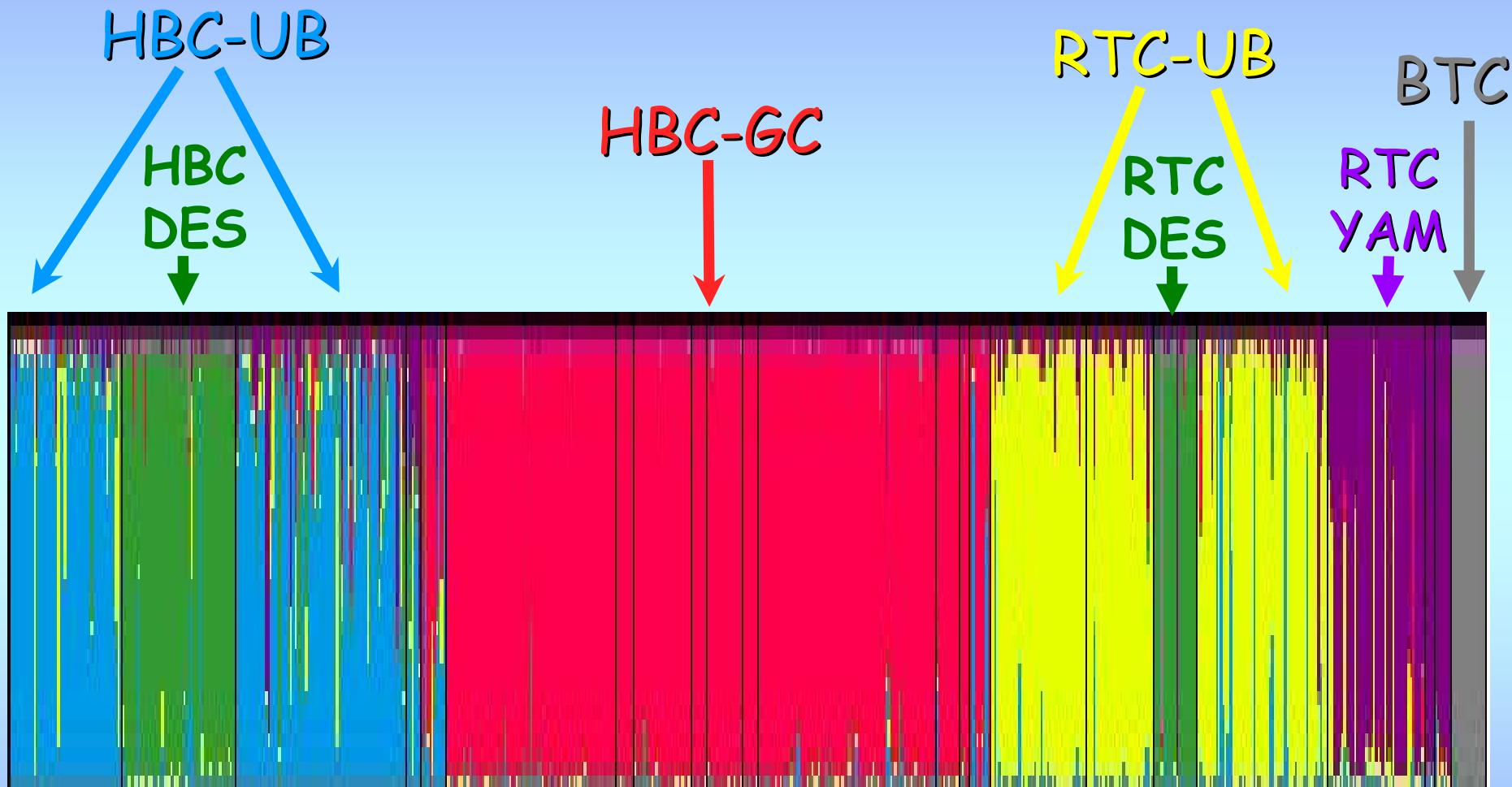
>> different from other Upper Basin RTC

Table 9 = GP7

BUT - **small sample size** for HBC Yampa R

msat - STRUCTURE: 6 Clusters

Fig 8



H	H	H	H	H	H	H	H	R	R	R	R	R	R
B	D	W	Y	3	L	S	K	W	1	D	W	Y	RRB
K	E	W	A	0	C	A	A	K	5	E	W	Y	YLB
R	S	C	M	M	R	H	N	V	M	S	C	A	DYT
								N	R			M	TCC

Conclusion (5)

❖ Bonytail

- admixture with HBC
 - >> historic signal of introgression
 - >> stronger signal Desolation Canyon

BAPs - Fig 5

msat - STRUCTURE: 5 Clusters

UB-H

UB-H

DC/BT

GC-H

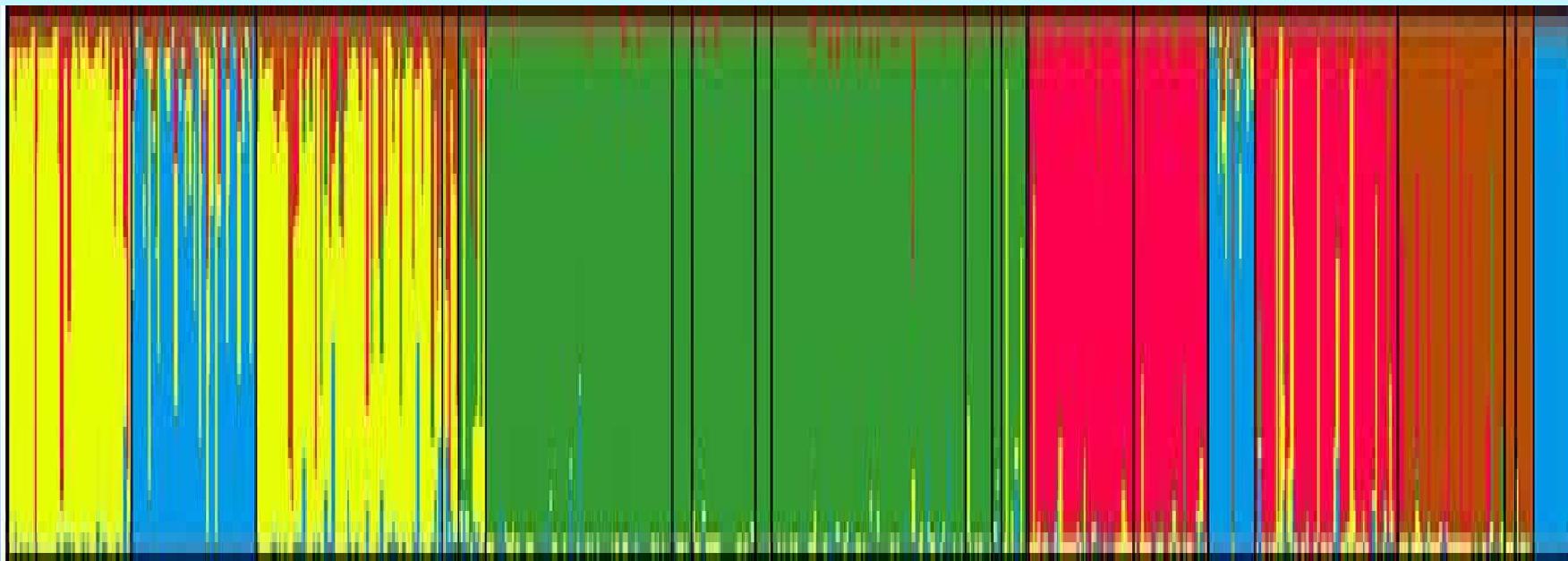
UB-R

UB-R

DC/BT

YR-R

DC/BT

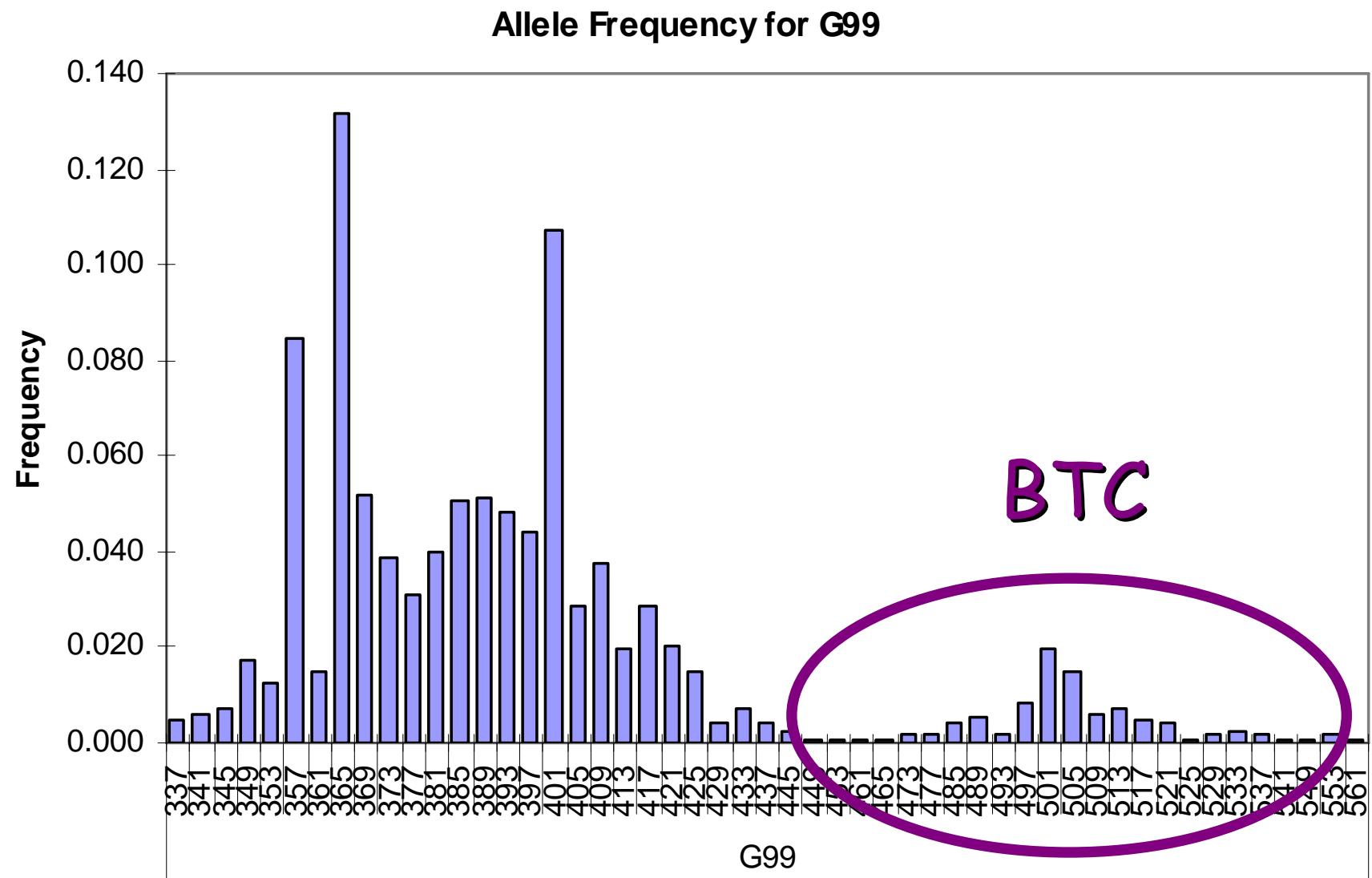


H	H	H	H	HH	H	HHH	H	HHH	R	R	R	R	R	R	B
B	D	W	Y	3	L	S	R	HKW	B	1	D	W	Y	Y	B
K	E	W	A	0	C	A	H	A	AAG	K	5	E	W	A	T
R	S	C	M	M	R	H	N	VNC	R	M	S	C	M	T	C

Msat Analysis - Admixture

- **Bonytail**
 - characteristic allele size ranges
 - some BTC in HBC
 - some HBC/RTC in BTC

Conclusion (5)



Compendium

- mtDNA reflects evolutionary history
 - admixture HBC / RTC
- msat also reflect morphology
 - distinct morphs
 - locality effect

Species still described morphologically

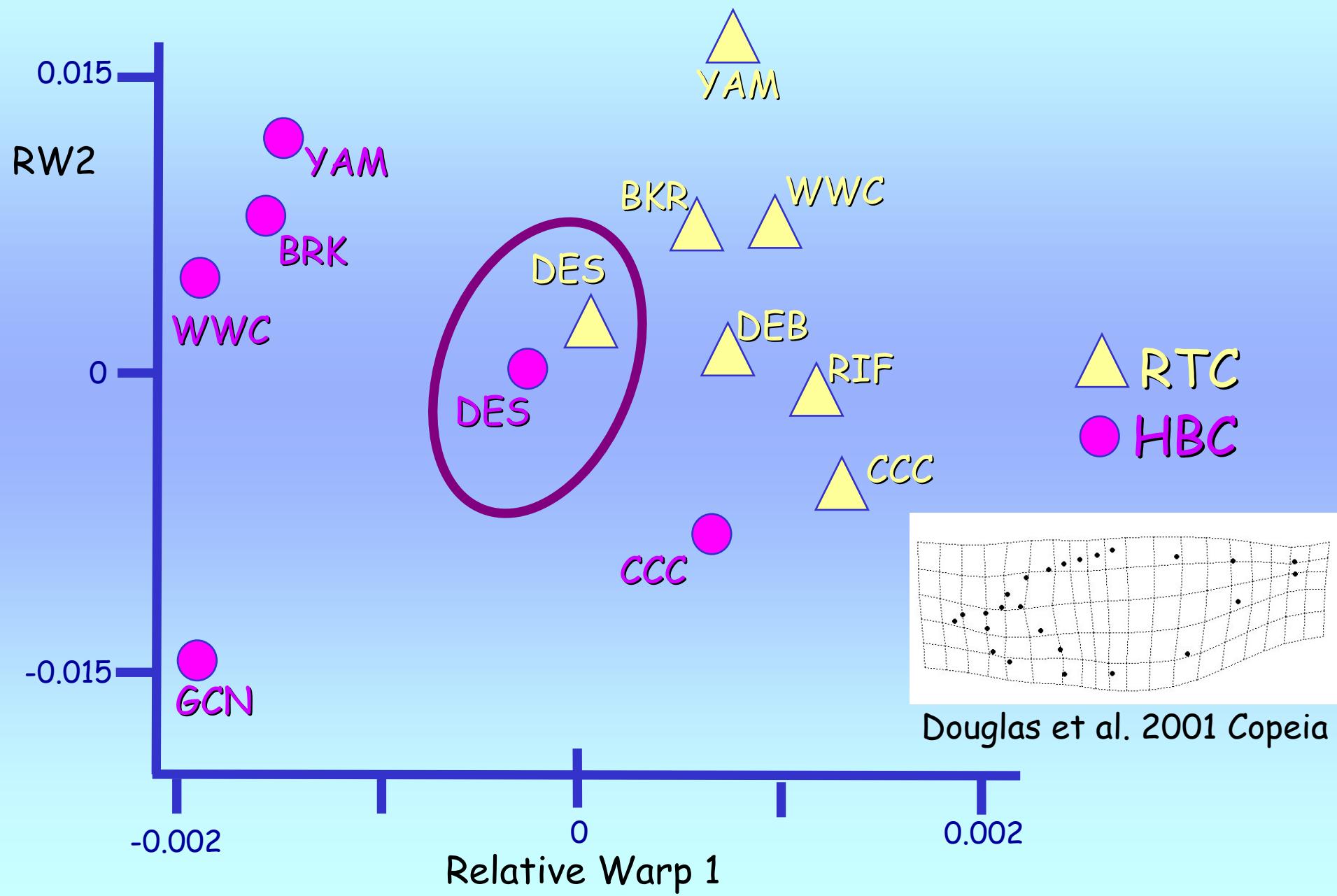
Morphology & Gila

- Morphometric Characters (museum specimen)
 - Discriminant Function Analysis
 - Adults: 95% allocated to species
 - Juveniles: 97% allocated to RTC or HBC/BTC



Douglas et al. 1998 TAFS

Shape Variation: Relative Warp Analysis



Thank You!

